LABORATORY EVALUATION OF *ASPERGILLUS* GALACTOMANNAN LATERAL FLOW ASSAYS

Dr Anja Ubbink

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in Medical Microbiology

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Declaration

I, Anja Ubbink, declare that this Research Report is my own work. It is being submitted for the Degree of Master of Medicine in Medical Microbiology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

Anja Ubbink

11/12/2023

Supervisors

1. Assoc Prof Vindana Chibabhai: MBBCh, FCPath Micro, DCH, DipHIVMan

   Signature:  Date: 12/12/2023

2. Dr Trusha Nana: FCPath Micro, MMed Micro, DTMH

   Signature:  Date: 12/12/2023

Format of report

This research report is written in a submissible format for the African Journal of Laboratory Medicine (AJLM).

Abstract

Background

Invasive aspergillosis diagnosis is based on a combination of clinical, radiological, and mycological factors, including the detection of *Aspergillus* galactomannan antigen in serum and bronchoalveolar lavage fluid (BALF). Lateral flow assays (LFA) introduced for rapid detection of galactomannan in serum and BALF include the IMMY sōna *Aspergillus* LFA (IMMY LFA) and the Dynamiker QuicGM™ *Aspergillus* Galactomannan Ag LFA (QuicGM LFA).

Objective

To evaluate the performance of the IMMY LFA and QuicGM LFA in South Africa.

Methods

Serum and BALF samples previously tested by Platelia BioRad *Aspergillus* GM-EIA were analysed using the two different LFAs. Percentage agreement and precision was assessed.

Results

Forty-six serum- and 13 BALF samples were tested using the IMMY GM LFA and 48 serum- and 6 BALF samples were tested using the QuicGM LFA. Using an optical density ≥0.5 as positive, results were compared to the BioRad *Aspergillus* GM-EIA. For the IMMY LFA, serum samples had a positive percent agreement (PPA) of 0% (0/1); negative percent agreement (NPA) of 91% (41/45) and overall percent agreement (OPA) of 89% (41/46). BALF samples had a PPA of 75% (3/4), NPA 50% (5/10) and OPA of 57% (8/14). For the QuicGM LFA, serum samples had a PPA 0% (0/3), NPA of 96% (43/45) and OPA of 90% (43/48). BALF samples had a PPA of 100% (1/1), NPA of 100% (5/5) and OPA of 100% (6/6). For the IMMY LFA, between-day reproducibility for 72% (13/18) and 63% (5/8) for serum

and BALF samples, respectively. Between-batch reproducibility was 89% (16/18) and 50% (4/8), respectively for serum and BALF samples. For the QuicGM LFA, between-day reproducibility was 75% (9/12) and 75% (3/4) for the serum and BALF samples, respectively. The between-batch reproducibility was 100% (8/8) for serum and 100% (3/3) for BALF.

Conclusion

A follow-up evaluation with a larger sample size utilizing clinical, radiological, and laboratory data is warranted to determine the assays' clinical utility.

What this study adds

Invasive aspergillosis is a life-threatening disease, where a prompt diagnosis improves outcome. Currently there is no *Aspergillus* galactomannan assay available in the South African state sector. This study evaluates two lateral flow assays for the detection of Galactomannan in South Africa.

Keywords

Invasive Aspergillosis; Galactomannan; Lateral flow assay

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List of Abbreviations

BALF	Bronchoalveolar Lavage Fluid
CE	Conformite Europeenne
COVID-19	Coronavirus Disease 2019
CSF	Cerebrospinal Fluid
EIA	Enzyme Immunoassay
EORTC/MSGERC	European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium
GM	Galactomannan
HIV	Human Immunodeficiency Virus
IA	Invasive Aspergillosis
IMMY LFA	IMMY sōna Aspergillus Lateral Flow Assay
LFA	Lateral Flow Assay
NHLS	National Health Laboratory Services
NPA	Negative Percent Agreement
ODI	Optical Density Index
OPA	Overall Percent Agreement
Platelia Biorad EIA	Platelia *Aspergillus* enzyme immunoassay
PPA	Positive Percent Agreement
QuicGM LFA	Dynamiker QuicGM™ Aspergillus Galactomannan Ag Lateral Flow Assay
TB	Tuberculosis
TPN	Total Parenteral Nutrition
WHO	World Health Organisation

1 Research Report

1.1 Introduction

Aspergillus fumigatus, a pathogen known to cause IA, is listed as a WHO critical priority fungal pathogen. The WHO priority pathogens are infections which are known to be challenging to diagnose and treat (1).

Aspergillus species are ubiquitous moulds and exposure to spores (conidia) is common and unavoidable (2). Invasive pulmonary aspergillosis develops in severely immunocompromised individuals, including those with neutropaenia, lung transplant recipients, the critically ill, patients on steroids, patients with chronic obstructive pulmonary disease, influenza, and coronavirus disease (COVID-19) (2,3). The population at risk is growing due to new groups of at-risk patients and an increasing number of immunocompromised individuals. IA has also been identified in immunocompetent hosts, without co-morbidities or mild chronic conditions (4). Morbidity and mortality associated with IA is high, with reported case fatality rates between 30 and 80% (5).

Although the true prevalence of IA is unknown in Africa, (6,7) an estimated 3885 cases are thought to occur in South Africa annually when considering at-risk group. South Africa has a high burden of human immunodeficiency virus (HIV), tuberculosis (TB) and poverty (8). HIV has been reported as a risk factor for IA from observational studies in Africa. Due to the unavailability of diagnostic tests for IA, the disease is unfortunately frequently undiagnosed or misdiagnosed as TB (6).

Establishing a prompt diagnosis is crucial in improving disease outcome (9) and a key aspect of an antifungal stewardship program (10). Diagnosis is, however, challenging and requires a

high index of suspicion. No single test confirms the diagnosis, and the required sampling is often invasive. A combination of histopathological, clinical, radiological and microbiological evidence, including the detection of galactomannan (GM), is required (2,11). Diagnostic assays with improved performance and rapid results, which can be performed in resource-limited settings are needed.

Culture-based methods for the diagnosis of IA are impeded by a slow turn-around time, limited sensitivity and significance of the culture may be difficult to determine due to the ubiquitous nature of *Aspergillus* species. In addition, high quality specimens for culture may require invasive sampling which is often impractical (12). Culture remains an essential part of the diagnosis for invasive fungal diseases as it allows for detection of other moulds and for susceptibility testing with the emergence of azole-resistant *Aspergillus fumigatus* (13).

Galactomannan is a polysaccharide secreted by *Aspergillus* species during fungal growth. It is a major constituent of the cell wall. The detection of GM in serum, plasma, bronchoalveolar lavage fluid (BALF) and cerebrospinal fluid is a non-culture-based method that assists with diagnosis of IA. (11,14).

The commercially available Platelia *Aspergillus* enzyme immunoassay (Platelia Biorad EIA) is only available at a limited number of laboratories and samples are usually tested in batches, increasing turn-around times. The complexity of an enzyme immunoassay makes it difficult to perform in a resource limited setting (15,16). It is currently the only GM detection test incorporated into the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) consensus definitions for the diagnosis of invasive fungal disease (17).

Recently, lateral flow assays (LFA) for the detection of GM have been developed. They are rapid, allow for single-sample testing and are promising for implementation in resource limited settings. These include two CE marked tests, the IMMY Sōna *Aspergillus* LFA (IMMY LFA) (IMMY, Norman, Oklahoma, USA) and the Dynamiker QuicGM Aspergillus Galactomannan Ag^{TM} LFA (QuicGM LFA) (Dynamiker, Tianjin, China) (14,18). Both these assays can give a result within an hour and because they do not need to be batched, this will not lead to increased turnaround times in low volume test settings as with the Platelia Biorad EIA.

Comparative large scale data from Africa for the performance of GM assays are not available (14,17). At the time of writing, there were no clinical performance studies evaluating the QuicGM LFA on PubMed and one study from Africa evaluating the IMMY LFA among haematological malignancy patients in Ghana (19).

The available LFAs utilize different monoclonal antibodies and test protocols, and the performance of these LFAs may differ from the Platelia Biorad EIA. Prior to implementation of a new test laboratories must be aware of its performance based on manufacturer and published evaluations. In addition, a verification of the assay performance in the hands of the implementing laboratory is required (14).

In this study, we evaluated the performance of two GM Ag LFAs, the IMMY Sōna *Aspergillus* galactomannan LFA and the Dynamiker QuicGM^{TM} LFA. Agreement and precision were also evaluated.

1.2 Methods

1.2.1 Ethics

Ethics approval was obtained from the University of Witwatersrand's Human Research Ethics Committee (Clearance certificate No. M220953). De-identified clinical samples were used for this study. No clinical information was included, and no human participants were involved.

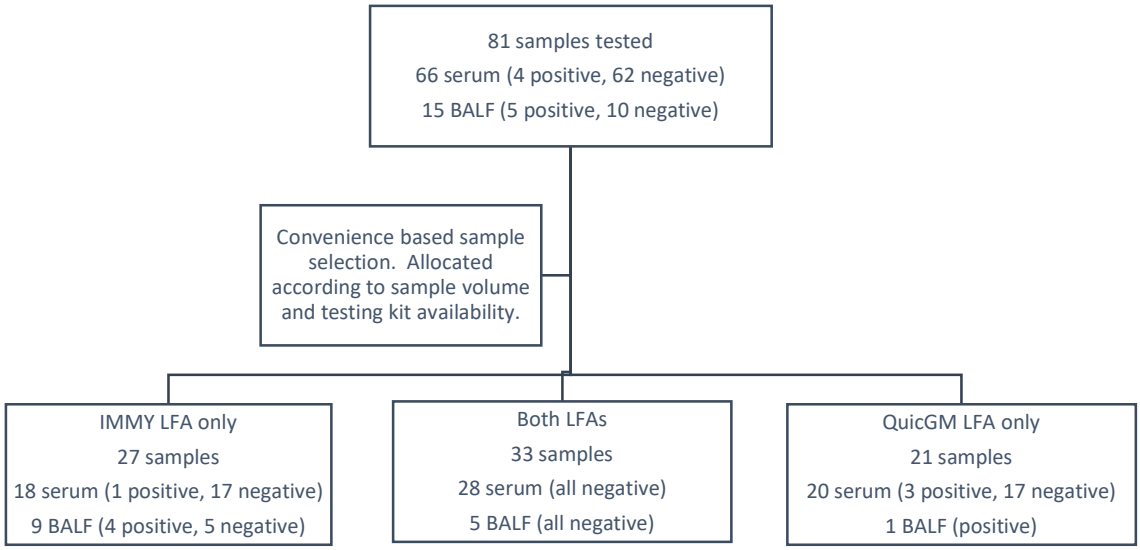
1.2.2 Setting

Samples were processed at the National Health Laboratory Service (NHLS) at the University of Witwatersrand Medical School.

1.2.3 Samples

De-identified clinical BALF and serum samples tested using the Platelia Biorad EIA for the detection of *Aspergillus* GM were obtained from a private microbiology laboratory service. The samples were stored at -70^{o} for up to 18 months and thawed and brought to room temperature for analysis. Convenience sampling was used based on sufficient residual volume to accommodate additional testing. Due to sample volume constraints, not all samples were tested using both kits and samples were randomly allocated to be tested with the IMMY LFA or QuicGM LFA as per test kit quantity availability as in Figure 1. Where sufficient sample volume was present, samples were tested using both kits.

Figure 1 Number of samples tested by each kit.



Investigators were blinded to original GM result until after testing. Seventy- two (62 serum and 10 BALF) negative samples and 9 (4 serum and 5 BALF) positive samples were included in the study (Table 1).

Table 1. Number of samples per ODI range when tested using the Platelia Biorad EIA

Result interpretation	ODI Range	Serum Samples	BALF samples
Negative	≤0.2	56	7
	0.3 – 0.4	6	3
Positive	0.5 – 0.7	2	1
	0.8 – 0.9	1	1
	≥ 1.0	1	3

Abbreviations:
ODI = Optical Density Index
BALF = Bronchoalveolar Lavage Fluid
Positive value ODI ≥ 0.5

1.2.4 Laboratory Processing

Serum and BALF samples as depicted in Figure 1 were tested by a single operator, following the manufacturers' instructions. Manufacturer provided positive and negative external controls were included in each batch.

IMMY GM LFA

All results were interpreted with a visual read-out as well as using the IMMY Cube Reader. The IMMY Cube Reader is an optional accessory to aid in the interpretation of the IMMY GM LFA. If the control line failed to develop, the test was regarded as invalid. The manufacturer recommends an optical density index (ODI) ≥ 0.5 as a positive result.

QuicGM LFA

This assay cannot be visually interpreted and requires the Fluorescence Immunoassay Analyzer for interpretation of the results. The optical density value is provided by the analyser. For quality control, the fluorescence analyser automatically calculates the signal of the control line on the test strip. The manufacturer recommends an optical density index (ODI) ≥ 0.5 as a positive result.

1.2.5 Precision testing

Samples were processed on consecutive days for between-day precision testing by a single operator. For the IMMY LFA, 26 samples (18 serum and 8 BALF) were tested on two consecutive days (due to limited kit availability). Only 1 positive BALF sample was included, the remaining samples were all negative. For the QuicGM LFA, fewer samples were tested but over 3 consecutive days as sample volume allowed. 16 samples (12 serum and 4 BALF) were analysed over two consecutive days and of those samples, 5 (2 serum and 3 BALF)

were tested for a third consecutive day. In this analysis, 3 serum samples were originally positive on the Platelia Biorad EIA, the rest of the samples were negative.

For between-batch precision testing samples were tested in duplicate, side by side, by a single operator, using two different kits with different batch numbers.

The same samples were used for both precision analyses. Since a gold standard assay was not available, precision was measured by consistent results between days and batches. For this analysis results were not compared to the original Platelia Biorad EIA result.

1.2.6 Data Analysis

Results were analysed using Microsoft Excel Software. Results from the IMMY LFA and QuicGM LFA were compared to the Platelia Biorad EIA results by calculating Percent Positive Agreement (PPA), Percent Negative Agreement (NPA) and Overall Percent Agreement (OPA). For the qualitative analysis 2x2 contingency tables for evaluation of the results were compiled for both kits as well as BALF and serum samples independently. Percentage agreement was calculated for the between-day and between-batch precision testing. The manufacturer's recommended optical density index (ODI) cut-off value of ≥ 0.5 for positive and < 0.5 for negative was used to interpret the ODI values.

1.3 Results

The agreement of the LFAs compared to the Platelia Biorad EIA is summarised in Table 2. Both assays did not detect GM in the positive serum samples. Table 3 compares the results of the positive samples. The ODI value for the serum samples ranged from 0.5 – 1.5 when tested on the Platelia Biorad EIA. In comparison, the positive BALF samples ranged from 0.5 to 5.3. The IMMY LFA agreed with 75% (3/4) positive BALF samples. Interestingly, it was the sample with the highest ODI value (ODI of 5.3) that tested negative using the IMMY LFA.

There was no residual sample leftover to retest this sample after unblinding. Overall, the ODI values of the LFAs of the positive samples tended to be lower when compared to the values of the Platelia Biorad EIA.

The NPA tended to be good (>90%) apart from BALF samples tested using the IMMY LFA. With further breakdown of the 5 BALF samples that tested positive using the IMMY kit and negative using the Platelia Biorad EIA, 4 of these samples had negative results when tested the next day or when using a different batch of the IMMY LFA and these discrepancies are evident in the precision testing results.

Table 2 Agreement of Lateral Flow Assays Compared to Platelia Biorad EIA

	Sample Type		**IMMY LFA** % n = 60	**QuicGM LFA** % n = 54
Percentage Agreement Compared to Platelia Biorad EIA	Serum	PPA	0% (0/1)	0% (0/3)
		NPA	91% (41/45)	96% (43/45)
		OPA	89% (41/46)	90% (43/48)
	BALF	PPA	75% (3/4)	100% (1/1)
		NPA	50% (5/10)	100% (5/5)
		OPA	57% (8/14)	100% (6/6)
	Total (serum + BALF)	PPA	60% (3/5)	25% (1/4)
		NPA	84% (46/55)	96% (48/50)
		OPA	82% (49/60)	91% (49/54)
Abbreviations: PPA = Positive Percent Agreement; NPA = Negative Percent Agreement; OPA = Overall Percentage Agreement; BALF = Bronchoalveolar Lavage Fluid				

Table 3 Subanalyses of Positive Samples

	Platelia Biorad EIA ODI	**IMMY LFA ODI**	**QuicGM LFA ODI**
Serum	0.9	0.4	
	0.7		0.3
	1.5		0.1
	0.5		0.3
BALF	5.3	0.4	

	5.2 0.9 0.5 2.8	0.6 0.7 0.6	 0.7

Abbreviations: EIA = Enzyme Immunoassay; ODI = Optical Density Index; LFA = Lateral Flow Assay; BALF = Bronchoalveolar lavage fluid

146

147 Thirty-three samples (28 serum and 5 BALF) were evaluated using both the IMMY LFA and
148 QuicGM LFA. This subset only consisted of negative samples when tested using the Platelia
149 Biorad EIA. The QuicGM LFA performed similarly with an NPA of 94% (31/33) compared to
150 the IMMY LFA with a NPA of 88% (29/33) for both serum and BALF samples pooled
151 together. Table 4 depicts the discrepant results during the precision testing. Different
152 samples had discrepant results when tested using the two LFAs.

153 *Table 4. Discrepant results of samples tested using both LFAs.*

Sample Type	Platelia Biorad EIA ODI	IMMY LFA ODI			QuicGM LFA ODI		
		Day 1 Lot no. 1	Day 2 Lot no. 1	Day 2 Lot no. 2	Day 1 Lot no. 1	Day 2 Lot no. 1	Day 2 Lot no. 2
BALF	0,2	0,52	0,18	0,53	0,15	0,11	0,13
BALF	0,2	0,86	1,68	1,27	0,16	0,16	0,13
BALF	0,0	0,77	0,05	0,07	0,17	0,18	0,19
Serum	0,1	0,59	0,30	0,14	0,10	Not repeated	Not repeated
Serum	0,1	0,10	Not repeated	Not repeated	1,03	Not repeated	0,34
Serum	0,1	0,11	Not repeated	Not repeated	0,99	Not repeated	Not repeated

Positive result ODI ≥ 0,5; Negative result ODI < 0,5

154

155 When assessed over two consecutive days, both LFAs performed sub-optimally as
156 summarised in Table 5. Seventy-two percent (13/18) serum and 63% (5/8) BALF samples
157 had the same qualitative result when tested using the IMMY LFA on two consecutive days.

All serum samples in this analysis were negative when tested by the Platelia Biorad EIA. Both serum and BALF samples with discrepant results, tested close to the cut-off ODI value of 0.5 (ODI values ranging from 0.51 to 0.78) when they tested positive.

Seventy-five percent (12/16) of samples had the same results when tested over two consecutive days using the QuicGM LFA. In this analysis, 25% (3/12) serum samples were positive when tested by the Platelia BioRad EIA and the remaining samples were negative. On day one of testing the serum samples, the QuicGM LFA did not detect GM in any of the positive samples. However, on Day 2 of testing one result was positive and the other gave a borderline ODI value of 0.49 and the last remained negative. Samples with residual volume were tested for a third day using the QuicGM LFA. Fifty percent (1/2) serum samples and 100% (3/3) BALF samples tested with the same quantitative result over 3 consecutive days.

For between-batch reproducibility, 89% (16/18) serum samples and 50% (4/8) BALF samples demonstrated reproducible results when tested with different batches of the IMMY LFA. The QuicGM LFA 100% (11/11) of samples were reproducible between batches, albeit fewer samples were tested.

Table 5 Precision Testing

	Sample Type	IMMY LFA % n	QuicGM LFA % n
Between-day Reproducibility			
2 consecutive days	Serum	72% (13/18)	75% (9/12)
	BALF	63% (5/8)	75% (3/4)
	Total (serum + BALF)	69% (18/26)	75% (12/16)
3 consecutive days	Serum	N/A[§]	50% (1/2)
	BALF	N/A[§]	100% (3/3)
	Total (serum + BALF)	N/A[§]	80% (4/5)
Between-batch Reproducibility	Serum	89% (16/18)	100% (8/8)
	BALF	50% (4/8)	100% (3/3)

	Total (serum + BALF)	77% (20/26)	100% (11/11)
Abbreviations: BALF = Bronchoalveolar Lavage Fluid; N/A = Not applicable § The IMMY LFA was tested on two consecutive days, and not for a third day			

174

175 The IMMY cube reader results correlated well with the visual readout results. Ninety-five
176 percent (104/110) of visual readout results correlated with the Cube Reader results. Three
177 of the discrepant results were reported as invalid by the Cube Reader but had a clear well-
178 developed control line and a valid result when visually inspected (2x negative result, 1x
179 positive result). To include these results in the analysis, the visual readout results were used
180 for the final interpretation of the IMMY LFA results. The remaining 3 results were negative
181 when visually inspected, however the Cube Reader reported the results as positive with
182 values close to the cut-off ODI value of 0.5 (the values were 0.51; 0.51 and 0.60).

183 ## 1.4 Discussion

184 In this study we compared the performance of two GM Ag LFAs, the IMMY LFA and
185 Dynamiker QuicGM ™ to the Platelia *Aspergillus* Ag EIA, using serum and BALF samples.
186 Verifying the accuracy of a new test for a disease with a large undiagnosed and unknown
187 burden is difficult and to our knowledge these assays have not been evaluated in the South
188 African population. In addition, this is the first study, to our knowledge, evaluating the
189 performance of both the IMMY and QuicGM LFAs together.

190 In the absence of associated clinical information, neither assay detected GM in the positive
191 serum samples. These samples originally had ODI values close to the cut-off of 0.5 with the
192 Platelia Biorad EIA. Overall, the ODI values of the LFAs of the positive samples tended to be
193 lower when compared to the values of the Platelia Biorad EIA. Studies have reported
194 conflicting results with regards to the stability of GM with storage. Pedrosa et al examined

the reproducibility of positive serum results when using the Platelia Biorad EIA. They observed a low reproducibility of positive results with more than half of the positive samples having negative results on retesting (20). Dufresne et al retested samples that were stored at -80^{o}C after the initial testing using the Platelia Biorad EIA and also reports a loss of positivity in 45% and 23% respectively, for both serum and BALF samples (21). Conversely, Wheat et al specifically examined the stability of galactomannan. Serum and BALF samples that were positive using the Platelia Biorad EIA were retested after storage at -20^{o}C for 5 years and 93% of the samples remained positive (22). Our samples had been stored short term at +4^{o}C, not for longer than the manufacturer recommended time and long term at -70^{o}C prior to testing.

The limited number of positive serum samples included in this study, did not allow for an adequate evaluation of the PPA the LFAs for serum. The NPA for serum samples on assessment of both LFAs were >90%.

The QuicGM LFA performed well with the BALF samples, with a 100% OPA with the Platelia Biorad EIA. Fewer samples and different samples were tested with the QuicGM LFA as opposed to the IMMY LFA and the results are not directly comparable between the two LFAs. The IMMY LFA performed poorly on the BALF samples. The reason for this is not clear. When the samples were retested with the same batch the next day as part of the precision testing, four of the BALF samples yielded results congruent with the Platelia Biorad EIA. Repeat testing of these samples was not possible due to insufficient sample volumes.

Both assays performed well when results were compared between batches, with the QuicGM LFA performing better than the IMMY LFA. Neither assay performed well when

tested over two consecutive days. Discrepant results had values close to the ODI cut-off value at 0.5.

Multiple factors may have contributed to discrepant results. Non-reproducibility has been reported in the repeat testing of false-positive samples when the patient does not have IA.

In a 900-bed tertiary facility in Switzerland, a retrospective analysis of the clinical information of patients whose samples turned negative on repetition found the variability of the test results is most probably due to false positivity. After introduction of the Platelia Biorad EIA at their institution, randomly selected positive and indeterminate serum samples were retested after the first measurement. The time to retesting was approximately 2 – 3 days and samples had either been stored at +4^{o}C or -20^{o}C. They found two clear trends amongst the retested samples. One subset of samples remained positive and the other subset yielded a very low ODI on repeat testing. The test results were correlated with the clinical diagnosis of IA. All samples (10/10) that remained positive, correlated with a diagnosis of possible, probable or proven IA. Out of the samples that turned negative, only 1 sample (1/11 samples from 10 patients) had a clinical diagnosis of probable aspergillosis (23).

This phenomenon was also examined in haematology patients at high risk of IA at a facility in the United Kingdom. As part of their routine neutropenic care pathway, at-risk patients are regularly tested for IA using *Aspergillus* polymerase chain reaction and *Aspergillus* GM using the Platelia Biorad EIA. Positive serum and plasma samples were correlated with the clinical probability of IA and classified into a case group (with possible, probable, or proven IA) or a control group (host factors with no clinical evidence of IA). In a comparison between the retesting of case samples versus the retesting of control samples, serum

indices declined significantly amongst the control samples, but no significant change was observed amongst the case samples (24).

Guigue et al also examined common causes of unreproducible positive results with the Platelia Biorad EIA for detection of *Aspergillus* GM and focussed on the initial preanalytical preparation steps of the assay as a cause of false positivity. The same serum samples were extracted by two different operators and then two tests (EIA) were performed on each extracted sample. Five-hundred and fifty consecutive serum samples were tested in this manner. Any unreproducible results were classified as either extraction unreproducible or ELISA unreproducible. When the samples with the unreproducible results were retested, the results turned negative for all except one which was inconclusive. The samples with reproducible results had similar GM ODIs upon retesting. The authors concluded unreproducible results are often due to operational factors which includes technical errors during the testing process, and could be due to a variety of factors, such as contamination, incorrect pipetting or improper storage of samples and could be present in the extraction step or a variation in the ELISA plate itself (25).

Causes of false positivity include presence of exogenous GM in the body which may be introduced as part of another product (e.g., gluconate or β-lactam antibiotics) and ingestion of GM containing food especially when the permeability of the gastrointestinal barrier is increased. There is also cross-reactivity with polysaccharides from closely related fungi (17).

Contamination is an important cause of false positive GM results and exogenous GM may be introduced pre-analytically or analytically. A facility in South Korea recently experienced high GM ODI values in serum from patients with no clinical evidence of IA. On review of the patient's medical records, the patients had received total parenteral nutritional (TPN)

products from the same manufacturer and the glucose component of the product had recently changed. The glucose components of the TPN product were diluted and tested for GM using the Platelia Biorad EIA with high positive results (26). In India, the sterile containers used to send BALF samples were found to be the cause of false positive results after an investigation into the reason for high GM levels in a population not suspected of having IA (27).

One study evaluating the IMMY LFA and Platelia Biorad EIA, found that haemolysed serum samples were either considered invalid by the cube reader or gave discrepant results, and found good agreement after the haemolysed samples were excluded from the study. Discrepancies were also seen in samples showing borderline GM values (28).

The IMMY cube-reader performed well when compared to the visual read-out of results. It provides a quantitative result and removes the subjectivity of visual interpretation. It was able to detect low levels of positivity when it was not visually possible. It performed comparably in a recent study, where results were read out by two independent readers and the Cube reader. The digital readout improved the diagnostic performance of the test (29). The use of digital readers is therefore critical to the success of the test.

For low resource settings, with inconsistent power supply, processing of samples requires a basic safety level two cabinet, heat block, centrifuge, and electricity for the digital readers. Therefore, electricity is still required for processing.

In this study we opted to use the manufacturer recommended cut-off OD value of ≥0.5 as positive. In 2020, the EORTC/MSGERC consensus definitions for invasive fungal diseases were updated. For the first time consensus cut-off values for GM were included and a cut-off of ≥ 1.0 was selected as positive for a single CSF, serum, plasma or BALF sample. A

combination of two low positive results of ≥0.7 ODI for serum or plasma, along with an ODI value in BALF ≥0.8 is also included as a criterion to suggest the presence of IA. The choice of threshold value impacts on the sensitivity and specificity of the test. These definitions, however, are not intended to direct patient care but for research purposes where a high degree of certainty of disease is required (11,17). In our setting, implementation of the test would primarily be to guide patient care. Only 4 samples in our study had GM values ≥1 and it was therefore not feasible to do an analysis using an ODI cut-off of 1.0.

There are several factors to consider when choosing the types of laboratory tests for aspergillosis diagnosis and when interpreting the results of these tests. In general, the sensitivity of GM testing is reduced in patients on mould-active antifungal agents. Additionally, the sensitivity of the GM testing varies depending on the underlying disease and specimen type (17). Two recent studies evaluating GM LFAs in patients with COVID-19, both demonstrated that low positive ODI values (0.5 – 0.8) in serum lacked specificity for COVID-19 associated pulmonary aspergillosis, and recommended borderline results should be confirmed by other microbiological tests (28,30).

Bronchoalveolar lavage fluid GM is more accurate than serum GM for diagnosing IA, especially in patients on mould-active agents. There are, however, higher false positive rates of GM in BALF, possibly due to *Aspergillus* colonisation and BALF requires invasive sampling (11,18). Hence, the results of GM testing should not be used as a stand-alone marker for aspergillosis. The pre-test probability of disease and results of other investigations must be integrated in the diagnostic decision.

The WHO has classified *Aspergillus fumigatus* as a critical priority fungal pathogen. A key step in addressing this threat is to improve diagnostic methods (1). However, diagnosis of IA

309 is challenging, especially in a resource limited setting. In a recent survey of the state of
310 clinical mycology in Africa, *Aspergillus* antigen detection was only available for 27% of
311 institutions locally and for 20% through an outsourced laboratory and none of the 8 South
312 African respondents performed an *Aspergillus* antigen EIA or equivalent (31). Another
313 recent survey found similar critical gaps in access to essential diagnostics for invasive fungal
314 infections in Africa. Aspergillus antigen detection was performed in as little as 8% of
315 participating countries. In South Africa the test was only privately available (32).
316 Improvement of diagnostic capacity is greatly needed in South Africa.

317 Improvement of diagnostic capacity for IA is a key aspect of antifungal stewardship. The use
318 of empirical antifungals in at-risk patients is common, due to the poor prognosis of these
319 infections when treatment is delayed. The accuracy and slow turnaround times of available
320 tests further hinder targeted treatment of these patients. The use of biomarkers, that are
321 accurate and with a fast turnaround time, will greatly improve antifungal stewardship
322 efforts (33).

323 The IMMY LFA performed well in previous evaluations. The IMMY LFA outperformed the
324 Platelia Biorad EIA in cancer population during a retrospective study done in New York City
325 (34). In another study, the IMMY LFA sensitivity was almost 97% and specificity 98%, with a
326 qualitative agreement of almost 90% when compared to the Platelia Biorad EIA (35). In an
327 evaluation of the QuicGM LFA in high-risk haematology patients, the results of the QuicGM
328 correlated well with the Platelia Biorad EIA with a Spearman's correlation coefficient of 0.80
329 (36). These tests therefore offer great promise in improving the diagnostic armamentarium
330 for IA.

Implementing a new test for a relatively rare disease is challenging. It is especially important to know the results are accurate and to date GM antigen testing has been poorly studied in the African context. We faced many challenges in this study including obtaining enough samples, the absence of clinical data, the need to test samples in an inter-laboratory setting and delays experienced during the COVID-19 pandemic.

Lateral flow assays for the detection of GM antigen are easy to perform and provide rapid results. Hence, these assays are well-suited for implementation in settings with limited resources.

1.4.1 Limitations

This study had many limitations. A single operator evaluated the tests. We tested samples initially analysed at a different laboratory using the Platelia Biorad Assay. Not all samples were tested by both kits due to limited kit and sample availability. The samples were stored at -70^{o}C and thawed and brought to room temperature for testing. Inter-laboratory variability in testing conditions, human error, environmental contamination, storage, and the patient's disease status impacted on the assays' reproducibility and perceived performance. Since convenience sampling was used, very few positive samples and very few BALF samples were included in this study. Positive samples are not readily available and invasive sampling for BALF is not routinely performed. We did not have clinical information available for any of the samples tested.

1.4.2 Conclusion

Expansion of diagnostic capability for IA is greatly needed in our community. The small sample size and variability in environmental conditions in this study limited the ability to make definitive conclusions regarding the performance of these assays. The challenges

experienced, and the limitations of our study demonstrate the dire need of funding of larger studies investing in diagnostics for IA in South Africa. Further testing is required to evaluate the performance of these assays. Further evaluation with a larger sample size, including more positive samples, as well as, inclusion of clinical, radiological, and histopathological data, and testing of samples in real-time, is recommended.

1.5 Acknowledgements

1.5.1 Competing interests

The authors have no competing interests to declare.

1.5.2 Sources of Support

The IMMY LFA kit and Cube Reader were provided by IMMY. The QuicGM™ LFA kit and Fluorescence Immunoassay Analyzer were sponsored by Dynamiker Biotechnology Co. Ltd. The manufacturers providing the assay did not have any input in the study design and did not participate in the study.

1.5.3 Disclaimer

The views expressed in this article are our own and not an official position of the University of Witwatersrand or National Health Laboratory Services.

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Appendix

2 Protocol

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

CANDIDATE'S SURNAME: **UBBINK**		FIRST NAME/S: **ANJA**	STUDENT NUMBER: **0603191A**
CURRENT QUALIFICATIONS: **MBChB, DipPEC, DTMH**			
TEL:	CELL: **060 304 4702**	E-MAIL: **anja.ubbink@gmail.com**	FAX: **N/A**
DEGREE FOR WHICH PROTOCOL IS BEING SUBMITTED: **Masters of Medicine (MMed) Microbiology**			
PART-TIME OR FULL-TIME: **FULL-TIME**			
FIRST REGISTERED FOR THIS DEGREE:	TERM : **SEPTEMBER**		YEAR: **2019**
DEPARTMENT: **Clinical Microbiology and Infectious Diseases**			
TITLE OF PROPOSED RESEARCH: **Laboratory Evaluation of Aspergillus Galactomannan Lateral Flow Assays**			
CANDIDATE'S SIGNATURE: A. Ubbink.			DATE: **30/08/2022**
SUPERVISOR 1 (NAME & SURNAME): **Dr Vindana Chibabhai**			**50**% Supervision
SUPERVISOR'S QUALIFICATIONS **MBBCh, FCPath Micro, MMed Micro, DCH, DipHIVMan**			
SUPERVISOR'S DEPARTMENT **Clinical Microbiology and Infectious Diseases**			
SUPERVISOR'S ADDRESS / TEL / E-MAIL: **083 564 9274 / vindana.chibabhai@nhls.ac.za**			
SUPERVISOR 2 (NAME & SURNAME): **Dr Trusha Nana**			**50**% Supervision
SUPERVISOR'S QUALIFICATIONS: **MBBCh, FCPath Micro, MMed Micro, DTMH**			
SUPERVISOR'S ADDRESS / TEL / E-MAIL: **083 641 6006 / trusha.nana@nhls.ac.za**			
SUPERVISOR 3 (NAME & SURNAME): **N/A**			% Supervision
SUPERVISOR'S QUALIFICATIONS			
SUPERVISOR'S ADDRESS / TEL / E-MAIL:			

SYNOPSIS OF RESEARCH:(Brief summary of proposed research project; between 200-300 words only; with sub-headings: an introduction and justification for study, aim/s, proposed methodology and expected outcome/s)
[Use reverse side of this page if more space is required]

Please see page 2 for synopsis of research.

WITS ETHICS NOT REQUIRED: Yes No WITS ETHICS PENDING: (Yes) No WITS ETHICS APPROVED: Yes No (circle appropriate symbol)* ***Please note the final human ethics clearance certificate or animal ethics certificate must be available prior to starting research**		IF Y SUPPLY ETHICS CLEARANCE CERTIFICATE AS ATTACHMENT AND INCLUDE ETHICS NUMBER HERE:
As supervisor/s, I/we confirm that I have read the protocol which has been submitted for assessment.		
SIGNATURE OF SUPERVISOR/S:	T. Nana	V. CHETTY BABHAI
SIGNATURE PG OFFICE STAFF	REGISTERED YES..... NO.....	STAMP

SYNOPSIS OF RESEARCH CONTINUED

INTRODUCTION

Invasive Pulmonary Aspergillosis (IPA) is an infection caused by *Aspergillus* species, a ubiquitous hyaline mould. The diagnosis of IPA is made based on a combination of clinical, radiological and laboratory tests, including the detection of *Aspergillus* galactomannan antigen in serum and broncho-alveolar lavage fluid (BALF).

JUSTIFICATION FOR STUDY

The National Health Laboratory Services currently do not offer an *Aspergillus* antigen test. When requested, the antigen test is referred to a private laboratory, resulting in delayed turn-around times and increased costs. The IMMY sōna- (IMMY LFA) and Dynamiker QuicGM™ (QuicGM™ LFA) *Aspergillus* galactomannan lateral flow assays are CE-marked, novel, rapid, low-cost antigen detection tests.

AIM

The aim of the study is to evaluate the IMMY LFA and QuicGM™ LFA against the Bio-Rad Platelia *Aspergillus* galactomannan antigen sandwich enzyme immunoassay (Bio-Rad Platelia EIA). We aim to determine the following:

1 Accuracy: Measurement of positive percentage agreement and negative percentage agreement of results.
2 Between day precision
3 Between batch precision
4 Evaluate the differences in performance when the European Organisation for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC)- and manufacturer recommended thresholds are applied to the quantitative results from the different assays.

PROPOSED METHODOLOGY

Serum and BALF samples tested by BioRad Platelia EIA have been obtained from Lancet Laboratory Services and are stored at -70°C. Thawed samples will be analysed using both IMMY LFA and QuicGM™ LFA. Results will be interpreted using two cut-off points - optical density value of ≥0.5 (manufacturer recommended) and ≥1.0 for serum and ≥0.8 for BALF (EORTC/MSGERC recommended). Between day and between batch precision will be determined.

EXPECTED OUTCOME

The two lateral flow assays have performed well in previous international evaluations.

11 March 2019/MP

Laboratory Evaluation of Aspergillus Galactomannan Lateral Flow Assays

Anja Ubbink

Student Number 0603101A

Masters of Medicine (MMed) Microbiology

Supervisors:

Dr Vindana Chibabhai

MBBCh, FCPath Micro, MMed Micro, DCH, DipHIVMan

Senior Pathologist, Clinical Microbiology Laboratory, Charlotte Maxeke Johannesburg Academic Hospital, National Health Laboratory Services, Johannesburg, South Africa

Senior Lecturer, Department of Clinical Microbiology and Infectious Diseases, University of the Witwatersrand, Johannesburg, South Africa

Dr Trusha Nana

MBBCh, FCPath Micro, MMed Micro, DTMH

Senior Pathologist, Clinical Microbiology Laboratory, Charlotte Maxeke Johannesburg Academic Hospital, National Health Laboratory Services, Johannesburg, South Africa

Lecturer, Department of Clinical Microbiology and Infectious Diseases, University of the Witwatersrand, Johannesburg, South Africa

2.1 Introduction

Aspergillus species is a ubiquitous hyaline mould. The *Aspergillus* conidia are frequently inhaled into airways and usually no disease results due to effective clearance of the spores. The inhaled fungal spores have the potential to cause infection. Syndromes range from colonisation; allergic responses to *Aspergillus* spp. such as allergic bronchopulmonary aspergillosis to semi-invasive and invasive conditions.(37)

The most severe manifestation is invasive aspergillosis (IA). It poses a significant threat to high-risk immunocompromised patients and is a major cause of morbidity and mortality in this population. The at-risk population include patients with prolonged neutropaenia, solid organ transplant, allogeneic hematopoietic stem cell transplant, inherited and acquired immunodeficiencies, corticosteroid use as well as critically ill patients with influenza and COVID pneumonia.(37–39) Globally, more than 300 000 cases are diagnosed annually with a 30 – 80% mortality rate. (5)

Early, reliable diagnosis of IA is crucial for prompt treatment and is a major challenge. Clinical symptoms are non-specific, especially in non-haematological patients admitted to intensive care units (ICU) with underlying diseases. No single definitive diagnostic test is available which reliably detects IA. Diagnosis relies on integration of clinical, radiological and microbiological factors. (37)

The European Organisation for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) revised criteria for defining invasive fungal infections require microbiological and/or histopathological evidence to diagnose proven infection. Different microbiological methods include culture, polymerase chain reaction (PCR) as well as detection of the Aspergillus galactomannan antigen.(11)

Culture based approaches are impeded by low sensitivities, especially in the early stages of infection and have a long turn-around time. *Aspergillus* species is a ubiquitous organism and frequently inhaled into the airway and usually effectively cleared by the immune system. Therefore, isolation of *Aspergillus* species from the airways is not always indicative of disease. Furthermore, acquisition of appropriate/good quality specimens may require invasive procedures, which is challenging in many patients and often not feasible.(12)

PCR can also be used for the detection of *Aspergillus* in tissue, bronchoalveolar lavage and blood.(11,40) Recently, it was included as a mycological criterion for probable invasive Aspergillosis in consensus guidelines for research studies.(11,40) Previously, there has been a lack of standardization among assays and a large variation in diagnostic performance across studies and different settings. *Aspergillus* PCR from blood shows particularly poor performance for the diagnosis of breakthrough infections in settings that use anti-fungal prophylaxis. With the availability of commercial assays, external quality control programmes, additional clinical validation, and standardized methodology, significant progress has been made. (40) However, these assays remain expensive and require specialised infrastructure and expertise not available in many settings.(41)

Due to the limitations of above diagnostic modalities, assays with improved performance and rapid results are necessary for early recognition and enablement of targeted therapy for invasive aspergillosis. There is a need for assays which can be performed in facilities with limited infrastructure. Non-culture-based techniques, including biomarkers, are promising diagnostic aids. Galactomannan (GM) is a major constituent of *Aspergillus* cell walls and is such a biomarker.(18)

Galactomannan makes up a major part of the cell wall of *Aspergillus* species. It is a polysaccharide that consists of a mannose backbone and a variable number of galactofuranose side chains. The fungus secretes these galactofuranose-containing side chains during growth.(17)

Enzyme-linked immunosorbent assays (ELISA) can detect the galactomannan polysaccharide and are used on serum or bronchoalveolar fluid specimens. Galactomannan ELISA is more sensitive than culture. The ELISA Galactomannan assay has been available for more than 20 years. (18)

The Bio-Rad Platelia *Aspergillus* galactomannan antigen sandwich enzyme immunoassay (Bio-Rad Platelia EIA) is such an assay that detects galactofuranose-containing molecules. It is a well-recognized test, FDA-approved and the only galactomannan antigen assay recommended in the EORTC consensus criteria for defining invasive fungal disease as an adjunct for the diagnosis of IA. It has been approved for testing serum and broncho-alveolar fluid.(17) Despite this, many clinicians globally do not have access to this test. There are many barriers to the implementation of this test, including long turnaround time, cost, requirement for batch testing and referral of the sample to a central laboratory. (11,18)

In settings where testing for galactomannan with ELISA is not achievable, the *Aspergillus* galactomannan lateral flow assays (GM-LFA) may be a viable option. These are novel, rapid, low-cost biomarker tests and include, the IMMY sōna *Aspergillus* GM Lateral Flow Assay (IMMY LFA) and the Dynamiker QuicGM™ *Aspergillus* Galactomannan Assay (QuicGM™ LFA). These assays can easily be performed in facilities with limited infrastructure, low sample throughputs and allow for rapid results. (17,18)

621 The galactomannan lateral flow assays are self-contained immunochromatographic
622 assays. It is used for qualitative and quantitative detection of *Aspergillus*
623 galactomannan from serum and bronchoalveolar lavage samples and is based on
624 the principle of lateral flow. If galactomannan is present in the sample, it binds to
625 galactomannan-specific antibodies conjugated to colloidal nano-gold forming an
626 antibody-antigen complex as it flows up the test strip through two testing zones. The
627 first zone has GM-specific antibodies (test line), the second zone has control
628 antibodies (control line) for quality control (18).

629 The IMMY LFA is Conformité Européenne (CE) marked. Recent studies with this
630 assay have reported reliable performance and good sensitivities on bronchoalveolar
631 fluid as well as serum for the diagnosis of invasive aspergillosis in haematology
632 patients.(29,42,43)

633 The QuicGM™ LFA was recently developed and is also CE markaed. Limited
634 performance data is available. One study found a concordance rate of >90%
635 between this assay and the Bio-Rad Platelia assay. 120 samples were included
636 (serum and BALF). All authors of the study were affiliated to Dynamiker Sub-Center
637 of Beijing Key Laboratory for Mechanisms Research and Precision Diagnosis of
638 Invasive Fungal Disease.(44)

639 The updated consensus definitions of invasive fungal disease from
640 EORTC/MSGERC were revised and published in 2020. Before this, these
641 consensus definitions were last updated in 2008. The 2008 definitions did not
642 contain thresholds for detecting Aspergillus galactomannan antigen as data were
643 insufficient at the time. The updated guidelines adopt different thresholds for
644 galactomannan for different specimen types. These thresholds differ from the

current manufacturer-recommended cut off values for the Bio-rad Platelia assay.(11,17)

The current manufacturer recommended cut-off value for a sample to be considered positive for galactomannan antigen in serum and BAL fluid is optical density ≥0.5 and for a negative result, the cut-off value is index < 0.5. (11,17)

The revised EORTC/MSGERC criteria advises the detection of any of the following to aid in the diagnosis of invasive aspergillosis: single serum or plasma galactomannan antigen ≥ 1.0; or BAL fluid ≥ 1.0; or single serum or plasma ≥ 0.7 and BAL fluid ≥ 0.8; or CSF ≥ 1.(11,17)

Importantly, the galactomannan cut- offs mentioned in the revised EORTC/MSGERC consensus documents are based on the Platelia *Aspergillus* assay. Insufficient evidence was available for incorporating novel *Aspergillus* antigen tests as their performance and corresponding cut-offs had been inadequately assessed. (17)

A pilot study was performed at Charlotte Maxeke Johannesburg Academic Hospital Microbiology Laboratory in 2021, evaluating the performance of the IMMY LFA against the Biorad Platelia assay. 19 Serum samples were processed using the IMMY LFA. Using an optical density cut-off of ≥0.5 as positive, the IMMY Aspergillus GM-LFA had a sensitivity of 33.3% (2/6), specificity of 92.3% (12/13) when evaluated against the BioRad Aspergillus GM-EIA. Between day reproducibility was 100% (8/8). The results showed the test may be promising. However, very few samples were included; sample type did not include broncho-alveolar lavage (BAL) fluid and few positive tests were available.(45)

For the diagnosis of invasive Aspergillosis diagnostic assays with improved performance, faster results and which can be performed in facilities with limited

infrastructure is needed. Currently there is no such test offered within the National Health Laboratory Services. The aim of this study is to evaluate the performance of two GM Ag LFAs, the IMMY LFA and Dynamiker QuicGM™ LFA in our local setting.

2.2 Study Objectives

The aim of the study is to evaluate the IMMY sōna *Aspergillus* Lateral Flow Assay (IMMY LFA) and the Dynamiker QuicGM™ *Aspergillus* Galactomannan Ag Lateral Flow Assay (QuicGM™ LFA) against the Platelia *Aspergillus* Ag assay with serum and bronchoalveolar lavage samples.

For the evaluation of the different assays the aim is to determine the following:

1. Accuracy: Measurement of positive agreement and negative agreement of results between the IMMY LFA, QuicGM™ LFA and Bio-Rad Platelia EIA assays for serum and bronchoalveolar lavage samples.
2. Between day precision: to confirm acceptable between-run variance
3. Between batch precision: to determine the variance of results of the same sample run with different batches of the assays
4. Evaluate the differences in performance when EORTC/MSGERC and manufacturer-recommended thresholds are applied to the quantitative results from the different assays.

2.3 Methods

2.3.1 Samples

De-identified clinical broncho-alveolar lavage fluid and serum samples tested using the Platelia Biorad assay for detection of the *Aspergillus* galactomannan antigen were obtained from Lancet Laboratory services. The samples represent both positive and negative results.

The samples are stored at -70^{o}C and will be thawed and brought to room temperature for analysis. For between day precision testing the samples will be stored at 2 – 8^{o}C and analysed within 72 hours.

Most guidelines recommend a minimum of 20 samples to be used for verification studies. (46,47) 50 serum samples and 15 broncho-alveolar lavage fluid samples will be tested using both the IMMY LFA and the QuicGM™ LFA.

To determine the precision of the tests:

- For between day precision the 20 serum samples and 10 bronchoalveloar lavage samples will be tested again on the following day.
- To determine between batch precision 20 serum samples and 10 bronchoalveolar samples will be tested in duplicate, side by side, using two different batch numbers of kits

The samples will be processed at Infection Control Laboratory Services (ICLS) at the University of Witwatersrand Medical School.

One analyst (the researcher) will be processing the samples. The analyst will not be aware of the previous results of the sample and the test result will be measured objectively using the cube reader (IMMY kit) and the AFS-1000 (QuicGM™ kit) to limit bias.

2.3.2 Safety Considerations

Appropriate personal protective equipment as well as in a biosafety cabinet level 2 will be used.

2.3.4 Materials needed

1) IMMY *Aspergillus* Antigen LFA kit
2) Cube Reader (for analysis of IMMY kit result)
3) Dynamiker Biotechnology QuicGM™ Galactomannan Ag LFA kit
4) AFS-1000 (reader for analysis of QuicGM™ kit)

5) Microcentrifuge screw-cap tubes 1.5 – 2 ml, capable of reaching 120°C
6) Disposable microcentrifuge tubes, test tubes or a microtitre plate
7) Pipettes (100µL, 1000µL) and disposable tips
8) Centrifuge (capable of reaching 14000 x g)
9) Vortex mixer
10) Heat block or water bath capable of reaching 120°C
11) Thermometer
12) Timer
13) Personal protective equipment
    a. Disposable gloves
    b. Protective glasses
    c. Lab coat
14) Biosafety cabinet level 2

2.3.5 Quality Controls

Processing of the samples will be performed in an accredited laboratory. Quality assurance of all equipment in the laboratory will be undertaken as part of standard operating procedure.

Both kits have an internal quality control line on the lateral flow assay strip. If the internal quality control fails, the test result will be regarded as invalid.

Manufacturer provided external quality controls will be used during every run of the test to ensure proper performance by the analyst and a proper functioning test kit.

An in-house external positive and negative control will be developed by using saline for the negative control and spiked saline with *Aspergillus* spp. culture for the positive control.

2.3.6 Testing Method

Samples will be processed by strictly adhering to the manufacturer's test procedure as recorded in the product package insert.

QuicGM™- Aspergillus Galactomannan Ag Lateral Flow Assay Test Procedure:

Sample treatment

- Add 300µL serum/BAL into screwcap microcentrifuge tubes
- Add 100µL sample treatment to above
- Screw the lid tightly and vortex for 10 seconds
- Using a heat block, heat the tubes at 110°C for 3-4 minutes
- Centrifuge the tubes at 10000 x g for 10 minutes
- Use the supernatant for performing the test below

Procedure

- Tests and samples need to be room temperature
- Place the test cassettes on a flat and clean bench
- Dispense 90 – 100µLof sample supernatant and slowly add into sample pad
- Read and record the results using the fluorescence immunoassay analyzer after 20 minutes

IMMY sōna *Aspergillus* Galactomannan LFA Test Procedure:

Sample Pretreatment:

- Place 300µL of serum or BAL into a heat resistant, screw cap microcentrifuge tube
- Add 100µL of sample pretreatment buffer to the same tube
- Screw lid tightly and vortex the sample
- Place tube in a heat block for 6-8 minutes at 120°C
- Immediately centrifuge the sample at 10000 – 14000 x g at room temperature

Procedure

- Add 120µL *Aspergillus* GM positive control into a clean tube

- Add 120µL *Aspergillus* GM LFA running buffer (negative control) into another clean tube
- Pipette 40µL of *Aspergillus* GM LFA running buffer into a separate clean tube or microwell
- Pipette 80µL of supernatant from pretreated serum / BAL each tube from above step
- Place one *Aspergillus* GM Lateral Flow test strip in each tube or microwell containing sample or control
- Allow the test to run for 30 minutes at room temperature
- Read and record the results within 10 minutes of completing the test, either visually or with the Cube reader

2.3.7 Performance Characteristics for Evaluation

The following performance characteristics will be determined for the IMMY LFA and the QuicGM™ LFA assay:

- Two sets of analyses will be done:
    - Using both a threshold of ≥ 0.5 (manufacturer recommended) and ≥ 1 for serum / ≥0.8 for BAL (EORTC/MSGERC recommended) overall agreement, positive percent agreement and negative percent agreement of positive and negative results of each assay compared to the Platelia Biorad EIA
    - Acceptable level of agreement will be >90%.
- Reproducibility of the tests
    - Between day precision
        - 20 serum samples and 10 BAL fluid samples will be run again on a different day. A combination of positive and negative samples will be used. Guidelines recommend a minimum of 2 positive and 2 negative samples over 5 days for qualitative assays and a minimum of 3 positive and 3 low positive samples for quantitative assays. (46)
    - Between batch precision

- 20 serum samples and 10 BAL fluid samples will be run using two different batch numbers and tested side by side. A combination of positive and negative samples will be used.
  - Acceptable level of reproducibility will be at least 90% agreement

2.3.8 Data Analysis

Descriptive analyses of the data will be performed using Microsoft Excel. Positive Percentage agreement and Negative Percentage agreement will be calculated. Positive agreement will be the proportion of the comparative method (Platelia Bio-Rad EIA) positive results in which the test method is positive. Negative agreement will be the proportion of comparative method (Platelia Bio-Rad EIA) results in which the test method is negative. A scatterplot will be performed for comparison.

2.3.8 Ethics

De-identified clinical samples will be used in this study and no human participants are involved. An ethics application to University of Witwatersrand's Human Research Ethics Committee is pending.

2.4 Timing

	APR 22	MAY 22	JUN 22	JUL 22	AUG 22	SEPT 22	OCT 22	NOV 22	DEC 22	JAN 23
Literature review										
Preparing protocol										
Protocol assessment										
Ethics application										
Sample processing and Collecting data										
Data analysis										
Writing up - thesis										

2.5 Funding

The IMMY LFA kit has been sponsored IMMY. The QuicGM™ LFA kit has been sponsored by Dynamiker Biotechology Co. Ltd. Funding for publication and stationery will be sought from the Department of Clinical Microbiology and Infectious Diseases, University of Witwatersrand.

2.6 Problems

The clinical serum and BAL fluid samples are stored at -70ºC for this study. They will be thawed and brought to room temperature for processing. For the QuicGM™ LFA kit, manufacturer's instructions allow sample storage of up to 72 hours at 2-8°C or below -20°C for up to 12 months, with a maximum of five repeated thawing and freezing. The IMMY LFA kit package insert allows for sample storage at 2-8°C for up to 5 days or for up to 2 weeks at <-20°C. A number of reports report a significant decline in serum indices after sample storage and retesting. (21,24,48,49) A different study supports the use of frozen serum or BAL specimens stored for at least 5 years at -20°C in the evaluation of diagnostic tests based on detection of galactomannan. (22) A very low-positive sample may become negative after storage. This may impact the results of the study. We acknowledge this as a limitation of the study.

A limited number of kits are available for testing purposes. It will not be possible to see if results can be replicated by using different samples or a different analyst.

While the prevalence of Invasive Aspergillus in South Africa is largely unknown, few samples positive for Galactomannan Antigen samples are available and many of the results are close to the cut-off point.

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1019 3 Ethics Clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Dr A Ubbink

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M220953

NAME: (Principal Investigator)	Dr A Ubbink
DEPARTMENT:	School of Pathology Dept of Clinical Microbiology & Infectious Diseases Medical School University
PROJECT TITLE:	*Laboratory evaluation of Aspergillus Galactomannan lateral flow assays*
DATE CONSIDERED:	2022/09/30
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Drs T Nana and V Chibabhai
APPROVED BY:	Dr N Naran, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	2022/11/09

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **September** and therefore reports and re-certification will be due in the month of **September** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator Date

1020 4 Approval of Title

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

06 January 2023
Person No: 0603191A
PAG

Dr A Ubbink
117 Norwood Garden Village
The Gardens 69 Hamlin Street
Abbotsford
2192
South Africa

Dear Dr Anja Ubbink

Master of Medicine in Microbiological Pathology: Approval of Title

We have pleasure in advising that your proposal entitled *Laboratory Evaluation of Aspergillus Galactomannan Lateral Flow Assays* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences